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ORIGINAL ARTICLE

Synthesis, characterization and antimicrobial activities of new bis-hydrazonothioxothiazolidinone derivatives

Ritu B. Dixit ^{a,*}, Ankit P. Bulsara ^a, Hemal B. Mehta ^a, Bharat C. Dixit ^b

^a Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, New Vallabh Vidyanagar 388 121, India

^b V.P. and R.P.T.P. Science College, Vallabh Vidyanagar 388 120, India

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 Antimicrobial activity

Abstract New bis-hydrazonothioxothiazolidinone derivatives based on 2-thioxothiazolidin-4-one were synthesized in good yields using a simplified experimental condition. The structure of synthesized compounds was established with the help of common physico-chemical analysis and various spectroscopic techniques like FT-IR, mass and ¹H NMR. The results of characterizations are in good agreement with the proposed structure of all the synthesized compounds. Further, the antimicrobial (antibacterial and antifungal) activities of all the synthesized derivatives were carried out against various species like *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus* by using agar-cup method. The results of antimicrobial screening showed that all the compounds have mild to moderate activity. However, some of the compounds (**3a**, **3b**, **3d**, **3e**, **3f**, **3g**, **3i** and **3j**) have shown better activity than the other.

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1. Introduction

Among the class of thiazolidines, 4-thiazolidones (2-thioxothiazolidin-4-ones) have been found to be important both

structurally as well as pharmacologically, due to the presence of >N–C–S linkage which imparts activity to the structure (Brown, 1961). Readily available thiazolidones, rhodanine (**1**) and *N*-methylrhodanine (**2**), as shown in Fig. 1 are reported (Maly, 1873; Andreasch, 1879; Liberman and Lange, 1881; Ghearghia, 1969) to have significant antitubercular activity (Hendricks, 1965) and used in the treatment of diabetic complications (Leskey and Zimenkovsky, 2004). These drugs are also under clinical trials as a potential thromimatic, antimicrobial, antiviral, anti-ischaemic, cardiovascular, anticancer and thrombolytic drugs (Leskey and Zimenkovsky, 2004).

Further, there are many reports regarding synthesis of 4-thiazolidones (Reddy and Krupadanam, 2010; Qiu et al., 2010; Verçoza et al., 2009; Tomasic and Masic, 2009), but only few reports are available in which bis-2-thioxothiazolidin-4-one derivatives have been studied (Gantla et al., 2009; Khanna

* Corresponding author.

E-mail address: ritsdixit@yahoo.co.in (R.B. Dixit).



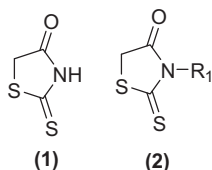


Figure 1 General structures of 4-thiazolidone derivatives.

et al., 2009; Jain et al., 2008, 2006; Kouznetsov et al., 2006). Looking to the aforementioned importance of 4-thiazolidone derivatives, we intended to synthesize new bis-hydrazonothioxothiazolidinone derivatives (**3a–3j**) using 2-thioxothiazolidin-4-one and diaryl diamines [diaminodiphenyl sulfone (DAPSON) (**4a**), diaminodiphenyl methane (DDM) (**4b**), diaminodiphenyl ether (DDE) (**4c**)] as well as nitro arylamines [3- and 4-nitroaniline (**4d** and **4e**)].

2. Experimental

2.1. Materials and methods

Decomposition temperature (uncorrected) for all the synthesized compounds was assessed using digital melting point apparatus. Elemental analysis for each compound was carried out using CARLO ERBA-1108 elemental analyzer. Mass spectral analysis was carried out using Thermo Fisher Scientific, MS-LCQ Fleet instrument by following ESI method at 70 eV. The FT-IR spectral analysis were performed using SHIMADZU 8400s forming pellet of substance with spectroscopic grade KBr in the range of frequency 400–4000 cm^{-1} . The ^1H NMR spectra were recorded on Bruker 400 MHz instrument using DMSO as a solvent as well as internal reference standard.

2.2. Synthesis of bis-phenyl bis-hydrazonothioxothiazolidinone derivatives (**3a–3f**)

2.2.1. 5,5'-(2,2'-(4,4'-Sulfonylbis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(2-thioxothiazolidin-4-one) **3a**

2.2.1.1. Tetra-azotization and coupling of diamines. Tetra-azotization of DAPSON was carried out by the method reported in the literature (Furniss et al., 2004). Accordingly, DAPSON (3 g, 12.08 mmol) was initially dispersed in 250 ml of aqueous solution of the conc. sulphuric acid (4.65 ml). The sodium nitrite solution was initially prepared by dissolving it (1.750 g, 25.37 mmol) in 20 ml of water. The above prepared solutions in beakers were kept in the ice bath at 0–5 $^{\circ}\text{C}$, then drop wise addition of sodium nitrite solution to the beaker containing suspension of DAPSON (**4a**) in sulphuric acid was carried out over 30 min by keeping temperature between 0 and 5 $^{\circ}\text{C}$. The tetra-azotized product (**5a**) obtained was immediately used for coupling reaction and coupled with 4-thiazolidone (**1**) in the ice bath at temperature 0–5 $^{\circ}\text{C}$ as reported in the literature (Singh et al., 1981). For that in 50 ml beaker, a solution was prepared by dissolving 4-thiazolidone (**1**) (3.379 g, 25.37 mmol) in 40 ml MeOH. It was added to the **5a** over a period of 20 min below temperature 5 $^{\circ}\text{C}$. The yellowish products obtained was washed with water and recrystallized from acetone to give bis-4-thiazolinone (**3a**) in 73% yield. The decomposition temperature was between 165 and 167 $^{\circ}\text{C}$ and color

was bright yellow. IR (KBr, cm^{-1}) 1239 (C=S), 1345 (S=O), 1755 (C=O), 3414 (N–H); ^1H NMR (400 MHz, DMSO- d_6), δ , ppm: 7.67 (d, 4H, J = 7.8 Hz, aromatic CH), 8.12 (d, 4H, J = 7.8 Hz, aromatic CH), 10.17 (s, 2H, –NH hydrazono), 12.61 (s, 2H, –NH rhodanine); MS (ESI, 70 eV), m/z : 537.97 (M^+); Anal. Calcd for $\text{C}_{18}\text{H}_{12}\text{N}_6\text{O}_4\text{S}_5$: C, 40.29; H, 2.55; N, 15.66; S, 29.88. Found: C, 40.45; H, 2.60; N, 15.61; S, 29.54.

Similarly, all other diphenylic derivatives of rhodanine (**3c**, **3e**) and *N*-methylrhodanine (**3b**, **3d**, **3f**) were prepared from appropriate diamines by the above method. Analytical data for **3b–3f** are as given below.

2.2.2. 5,5'-(2,2'-(4,4'-Sulfonylbis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(3-methyl-2-thioxothiazolidin-4-one) **3b**

Yield: 69%; decomposition temperature 200–204 $^{\circ}\text{C}$; color: Brownish orange; IR (KBr, cm^{-1}) 1711 (C=O), 1268 (C=S), 1308 (S=O), 2920 (C–H_{ali}), 3459 (N–H); ^1H NMR (400 MHz, DMSO- d_6), δ , ppm: 3.27 (s, 6H, 2 N(CH₃)), 7.64 (d, 4H, J = 8.0 Hz, aromatic CH), 8.05 (d, 4H, J = 8.0 Hz, aromatic CH), 9.96 (s, 2H, –NH hydrazono); MS (ESI, 70 eV), m/z : 565.98 (M^+); Anal. Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_6\text{O}_4\text{S}_5$: C, 42.54; H, 2.86; N, 14.88; S, 28.39. Found: C, 42.14; H, 3.01; N, 15.14; S, 28.23.

2.2.3. 5,5'-(2,2'-(4,4'-Methylenebis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(2-thioxothiazolidin-4-one) **3c**

Yield: 78%; decomposition temperature 171–175 $^{\circ}\text{C}$; color: Pale yellow; IR (KBr, cm^{-1}) 1256 (C=S), 1765 (C=O), 2880 (C–H_{ali}), 3403 (N–H); ^1H NMR (400 MHz, DMSO- d_6), δ , ppm: 3.96 (s, 2H, Ph-CH₂-Ph), 7.59 (d, 4H, J = 7.8 Hz, aromatic CH), 8.02 (d, 4H, J = 7.8 Hz, aromatic CH), 10.05 (s, 2H, –NH hydrazono), 12.46 (s, 2H, –NH rhodanine); MS (ESI, 70 eV), m/z : 487.01 (M^+); Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{N}_6\text{O}_2\text{S}_4$: C, 46.90; H, 2.90; N, 17.27; S, 26.36. Found: C, 46.79; H, 2.88; N, 17.14; S, 26.27.

2.2.4. 5,5'-(2,2'-(4,4'-Methylenebis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(3-methyl-2-thioxothiazolidin-4-one) **3d**

Yield: 72%; decomposition temperature 193–198 $^{\circ}\text{C}$; color: Dull yellow; IR (KBr, cm^{-1}) 1274 (C=S), 1706 (C=O), 2898 (C–H_{ali}), 3386 (N–H); ^1H -NMR (400 MHz, DMSO- d_6), δ , ppm: 3.22 (s, 6H, 2 N(CH₃)), 3.88 (s, 2H, Ph-CH₂-Ph), 7.60 (d, 4H, J = 7.6 Hz, aromatic CH), 7.92 (d, 4H, J = 7.6 Hz, aromatic CH), 9.87 (s, 2H, –NH hydrazono); MS (ESI, 70 eV), m/z : 515.04 (M^+); Anal. Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_6\text{O}_2\text{S}_4$: C, 49.01; H, 3.53; N, 16.33; S, 24.92. Found: C, 49.25; H, 3.48; N, 16.46; S, 25.09.

2.2.5. 5,5'-(2,2'-(4,4'-Oxybis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(2-thioxothiazolidin-4-one) **3e**

Yield: 80%; decomposition temperature 180–186 $^{\circ}\text{C}$; color: Yellowish orange; IR (KBr, cm^{-1}) 1201 (C–O–C), 1280 (C=S), 1735 (C=O), 3426 (N–H); ^1H NMR (400 MHz, DMSO- d_6), δ , ppm: 7.63 (d, 4H, J = 8.0 Hz, aromatic CH), 7.97 (d, 4H, J = 8.0 Hz, aromatic CH), 10.11 (s, 2H, –NH hydrazono); 12.54 (s, 2H, –NH rhodanine); MS (ESI, 70 eV), m/z : 488.99 (M^+); Anal. Calcd for $\text{C}_{18}\text{H}_{12}\text{N}_6\text{O}_3\text{S}_4$: C, 44.25; H, 2.48; N, 17.20; S, 26.25. Found: C, 44.11; H, 2.21; N, 17.08; S, 26.05.

2.2.6. 5,5'-(2,2'-(4,4'-Oxybis(4,1-phenylene))bis(hydrazin-2-yl-1-ylidene))bis(3-methyl-2-thioxothiazolidin-4-one) 3f
Yield: 68%; decomposition temperature 185–190 °C; color: Dark orange; IR (KBr, cm^{-1}) 1175 (C–O–C), 1293 (C=S), 1745 (C=O), 2935 (C–H_{ali}), 3469 (N–H); ¹H NMR (400 MHz, DMSO-*d*₆), δ , ppm: 3.34 (s, 6H, 2 N(CH₃)), 7.63 (d, 4H, *J* = 8.2 Hz, aromatic CH), 7.99 (d, 4H, *J* = 8.2 Hz, aromatic CH), 9.95 (s, 2H, –NH hydrazone); MS (ESI, 70 eV), *m/z*: 517.02 (M⁺); Anal. Calcd for C₂₀H₁₆N₆O₃S₄: C, 46.50; H, 3.12; N, 16.27; S, 24.83. Found: C, 46.38; H, 3.01; N, 16.21; S, 24.62.

2.3. Synthesis of mono-phenyl bis-hydrazoneothioxothiazolidinone derivatives (3g–3j)

2.3.1. 5,5'-(2,2'-(1,3-Phenylene))bis(hydrazin-2-yl-1-ylidene))-bis(2-thioxothiazolidin-4-one) 3g

2.3.1.1. Diazotization and coupling of mono amine. Diazotization of 3-nitroaniline (**4d**) was carried out by the method reported in the literature (Furniss et al., 2004). Accordingly, **4d** (3 g, 21.72 mmol) was initially dissolved partially in the concd sulphuric acid (4.33 ml). The sodium nitrite solution was prepared by dissolving it (1.709 g, 24.7 mmol) in 30 ml water. The above prepared solutions were kept in ice bath for 30 min and then NaNO₂ solution was added drop wise to the beaker containing acidic solution of amine under continuous stirring over the period of 20 min, while maintaining the temperature between 0 and 5 °C. The diazonium salt of **4d** generated was immediately used for coupling reaction. The diazonium salt of **4d** was coupled with 4-thiazolidone derivatives (rhodanine) (**1**) as carried out previously (Singh et al., 1981). Accordingly, compound **1** (3.62 g, 37.5 mmol) was dissolved in 40 ml MeOH and was added drop wise to the solution of diazonium salt over a period of 30 min under continuous stirring while maintaining the temperature between 0 and 5 °C. After completion of addition the beaker was kept under stirring in the ice bath for 30 min. The product (**5g**) thus obtained was washed with water and was recrystallized from acetone.

2.3.1.2. Reduction of nitro derivative 5g. Reduction of **5g** was carried out by the method reported in the literature (Ahluwalia and Aggarwal, 2004). Accordingly, nitro compound (**5g**) (2 g, 7.08 mmol) was suspended in 100 ml water and was heated to boiling. To the above reaction mixture sodium polysulphide (20 ml) solution in water was added drop wise over a period of 30 min and was boiled for 30 min. The reaction mixture was then allowed to cool and the crushed ice was added to it. The product obtained was filtered and washed with cold water. To the solid product obtained, 25 ml water and 10 ml concd HCl was added and was boiled for 10 min. The hot solution was filtered off and solid residue was rejected. The filtrate was collected in 500 ml beaker and 40% ammonia solution was added to it under continuous stirring, till the solution became basic. The solid product thus obtained (**6g**) was filtered, washed with water and recrystallized from acetone.

2.3.1.3. Diazotization and coupling of 6g. Compound **6g** was diazotized to obtained diazonium salt by following the method reported in the literature (Furniss et al., 2004). Accordingly, **6g** (1.5 g, 5.944 mmol) was initially dissolved partially in 25 ml aqueous solution of concd sulphuric acid (2 ml). The sodium

nitrite solution was prepared by dissolving 0.5 g (6.5 mmol) of it in 30 ml water. The above prepared solutions were kept in an ice bath for 10 min and then NaNO₂ solution was added drop wise to the beaker containing acidic solution of amine under continuous stirring over the period of 20 min by keeping the temperature below 5 °C. The diazonium salt of amine generated was used immediately for coupling reaction. The diazonium salt of amine was coupled with 4-thiazolidone derivative (**1**) in ice bath at 0–5 °C temperature by following the method reported (Singh et al., 1981). Accordingly, 0.870 g (12.65 mmol) of compound **1** was taken in 30 ml MeOH and was added drop wise to the solution of diazonium salt of **6g** over a period of 30 min under continuous stirring. After completion of addition, reaction mixture was further stirred for 30 min in the ice bath on magnetic stirrer. The bis-hydrazoneothioxothiazolidinone product (**3g**) thus obtained was filtered, washed with water and recrystallized from ethanol. The color, decomposition temperature and yield for the produced compounds were yellowish brown, 220–223 °C and 43%, respectively. IR (KBr, cm^{-1}) 1293 (C=S), 1731 (C=O), 3393 (N–H); ¹H NMR (400 MHz, DMSO-*d*₆), δ , ppm: 7.08 (t, 1H, *J* = 7.6 Hz, aromatic CH), 7.32 (dd, 2H, *J* = 7.6 Hz, 1.2 Hz, aromatic CH), 7.98 (s, 1H, aromatic CH), 10.46 (s, 2H, –NH hydrazone), 12.34 (s, 2H, –NH rhodanine); MS (ESI, 70 eV), *m/z*: 397.06 (M⁺); Anal. Calcd for C₁₂H₈N₆O₂S₄: C, 36.35; H, 2.03; N, 21.20; S, 32.35. Found: C, 36.21; H, 2.35; N, 20.78; S, 32.48.

All other monophenyl derivatives of rhodanine and *N*-methylerhodanine (**3h**, **3i** and **3j**) were prepared by following the above method. Analytical data for them are given below:

2.3.2. 5,5'-(2,2'-(1,3-Phenylene))bis(hydrazin-2-yl-1-ylidene))-bis(3-methyl-2-thioxothiazolidin-4-one) 3h

Yield: 35%; decomposition temperature 200–205 °C; color: Chocolate brown; IR (KBr, cm^{-1}) 1241 (C=S), 1745 (C=O), 2954 (C–H_{ali}), 3372 (N–H); ¹H-NMR (400 MHz, DMSO-*d*₆), δ , ppm: 3.08 (s, 6H, 2 N(CH₃)), 7.02 (t, 1H, *J* = 7.8 Hz, aromatic CH), 7.22 (dd, 2H, *J* = 7.8 Hz, 1.4 Hz, aromatic CH), 7.91 (s, 1H, aromatic CH), 10.38 (s, 2H, –NH hydrazone); MS (ESI, 70 eV), *m/z*: 424.99 (M⁺); Anal. Calcd for C₁₄H₁₂N₆O₂S₄: C, 39.61; H, 2.85; N, 19.80; S, 30.21. Found: C, 39.41; H, 2.55; N, 19.80; S, 29.14.

2.3.3. 5,5'-(2,2'-(1,4-Phenylene))bis(hydrazin-2-yl-1-ylidene))-bis(2-thioxothiazolidin-4-one) 3i

Yield: 39%; decomposition temperature 230–233 °C; color: Brownish orange; IR (KBr, cm^{-1}) 1222 (C=S), 1732 (C=O), 3363 (N–H); ¹H NMR (400 MHz, DMSO-*d*₆), δ , ppm: 7.28 (s, 4H, aromatic CH), 10.39 (s, 2H, –NH hydrazone), 12.29 (s, 2H, –NH rhodanine); MS (ESI, 70 eV), *m/z*: 396.96 (M⁺); Anal. Calcd for C₁₂H₈N₆O₂S₄: C, 36.35; H, 2.23; N, 20.96; S, 32.35. Found: C, 36.52; H, 2.03; N, 21.20; S, 32.48.

2.3.4. 5,5'-(2,2'-(1,4-Phenylene))bis(hydrazin-2-yl-1-ylidene))-bis(3-methyl-2-thioxothiazolidin-4-one) 3j

Yield: 31%; decomposition temperature 202–208 °C; color: Bright orange; IR (KBr, cm^{-1}) 1254 (C=S), 1743 (C=O), 2914 (C–H_{ali}), 3448 (N–H); ¹H-NMR (400 MHz, DMSO-*d*₆), δ , ppm: 3.19 (s, 6H, 2 N(CH₃)), 7.22 (s, 2H, aromatic CH); 10.28 (s, 2H, –NH hydrazone); MS (ESI, 70 eV), *m/z*: 424.99 (M⁺); Anal. Calcd for C₁₄H₁₂N₆O₂S₄: C, 39.61; H,

2.85; N, 19.80; S, 30.21. Found: C, 39.51; H, 2.25; N, 19.76; S, 30.21.

2.4. Antimicrobial studies for synthesized compounds **3a–3j**

The antimicrobial (antibacterial and antifungal) activities of all the synthesized compounds (**3a–3j**) were carried out against various species like *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus* by using agar-cup method (Perry and Stalay, 1997; Atlas, 1999). Benzylpenicillin (anti bacterial) and Imidil (antifungal) were used as the standard drugs and solvent DMSO as a control. The results of the zone of inhibition (in mm) for all the synthesized compounds **3a–3j** along with the control and standard are summarized in Table 1.

3. Results and discussion

3.1. Preparation of entitled compounds **3a–3j**

To achieve the target molecules, one mole of diaryl diamines were tetra-azotized and coupled with two moles of rhodanine

or *N*-metylerhodanine below 5 °C temperature to give **3a–3f** in good yields (65–75%) as shown in Scheme 1. However, diamines used were in the form of dispersion in acidic solution during tetra-azotization. Further, **3g–3j** were synthesized from nitro arylamines in good yields as shown in Scheme 2. Initially, nitro arylamines were diazotized and coupled with one mole of rhodanine or *N*-metylerhodanine to obtain nitro derivatives of 2-thioxothiazolidine-4-one (**5g–5j**). The reduction of nitro derivatives (**5g–5j**) was carried out with the help of sodium polysulphide to produce the corresponding amines (**6g–6j**), further it was diazotized and coupled with one mole of **1** or **2** (Fig. 1) in a similar fashion. The overall yield of 55% was obtained for the isolated products (**3g–3j**).

The progress of the reaction was continuously monitored by TLC using hexane–EtOAc–MeOH (80:15:5) as mobile phase and by viewing the spot under UV light. The appearance of all the synthesized compounds was from yellow to reddish-brown.

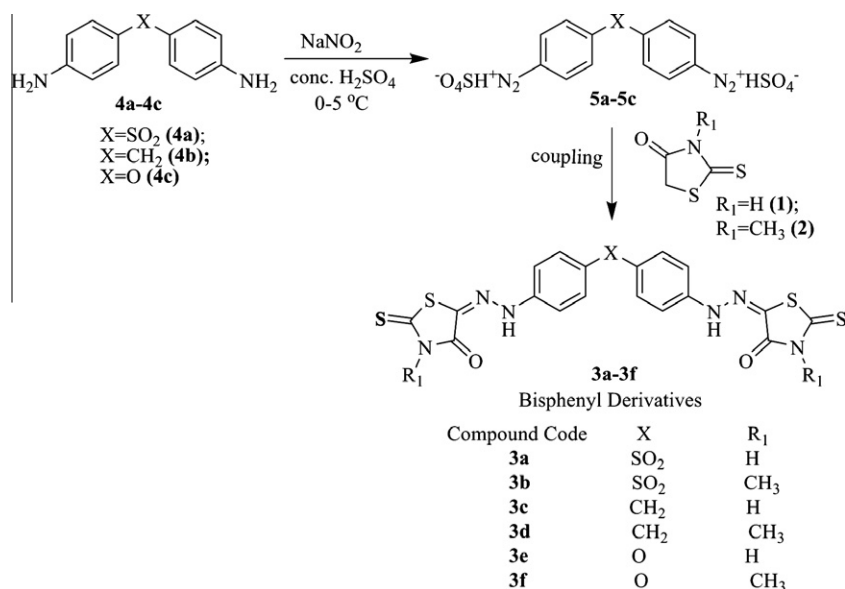
3.2. Spectral analysis of synthesized compounds **3a–3j**

The results of Mass spectral analysis and elemental analysis are in good agreement with the proposed molecular formula of the respective compound. The FT-IR spectra of all the derivatives (**3a–3j**) showed characteristic vibrations as an evidence of all the functionalities present. It showed some significant characteristic bands in the region of 1705–1788 cm^{−1} for C=O group. Further, for –NH– group vibrational and rotational movements gave bands at 3363–3469 cm^{−1}, respectively. Stretching bands due to C=N, SO₂, –O–, C=S groups appeared at 1645–1660, 1290–1305, 1050–1065 and 1190–1270 cm^{−1}, respectively.

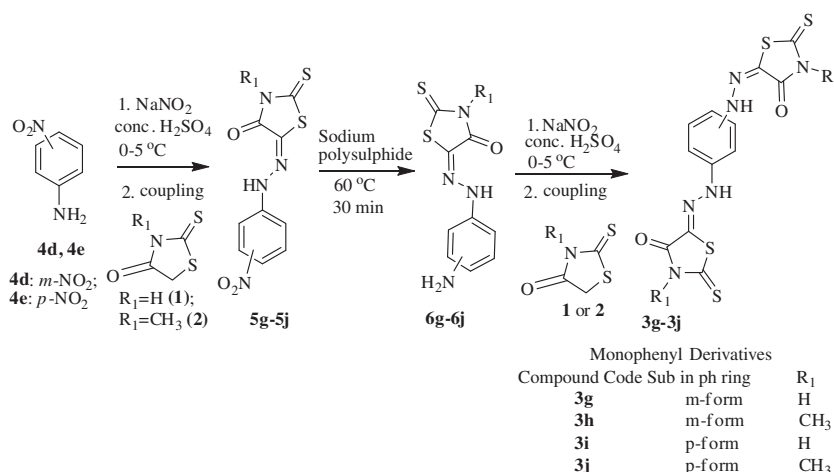
Structure of all the produced compounds was confirmed by ¹H NMR spectral analysis, which shows the characteristic values. Two aliphatic protons of Ar–CH₂–Ar appeared as a singlet at 3.96 δ ppm, while that of *N*-metylrhodanine containing derivatives showed a singlet at 3.22 δ ppm for six protons (i.e. of two N–CH₃). The protons of the aromatic ring appeared in the region of 7.02–8.12 δ ppm. The signals due to protons of secondary amines were also distinguished into

Table 1 Antimicrobial activity of entitled compounds (**3a–3j**).

Sr. No.	Compound code	Bacteria		Fungi	
		<i>B. subtilis</i>	<i>E. coli</i>	<i>A. niger</i>	<i>A. Flavus</i>
1	Control	5	5	–	–
2	3a	7	9	16	14
3	3b	16	10	8	12
4	3c	10	8	13	8
5	3d	9	9	12	16
6	3e	17	15	11	7
7	3f	14	13	10	10
8	3g	15	11	8	8
9	3h	11	9	11	11
10	3i	10	12	16	16
11	3j	17	11	14	11
12	Benzylpenicillin	20	20	–	–
13	Imidil	–	–	20	20



Scheme 1 Synthesis of bisphenyl bishydrazonothioxothiazolidinones (**3a–3f**).



Scheme 2 Synthesis of monophenyl bishydrazonothioxothiazolidinones (**3g–3j**).

two types; amongst these two, protons of hydrazono group(s) gave a singlet in higher field region (between 9.87 and 10.46 δ ppm) and little bit of broadening was observed in the peak, but protons attached to the nitrogen of rhodanine gave a singlet in the down field region (between 12.29 and 12.61 δ ppm).

3.3. Antimicrobial studies for **3a–3j**

The antimicrobial (antibacterial and antifungal) activities of all the synthesized compounds were carried out against various species as discussed in the experimental section using agar-cup method. Benzylpenicillin (anti bacterial) and Imidil (antifungal) were used as the standard drugs and solvent DMSO as a control. The results that are summarized in Table 1, reveal that amongst all the compounds, **3b**, **3e**, **3f**, **3g** and **3j** have good antibacterial activity, where as compounds **3a**, **3b**, **3d**, **3i** and **3j** have good antifungal activity than other compounds.

4. Conclusion

In summary, a very simple and efficient method was used to prepare newer bis-hydrazonothioxothiazolidinone derivatives. The results of antimicrobial screening have shown that all the tested compounds have mild to moderate activity against each species used. However, among the tested compounds, **3b** and **3j** have shown better antibacterial as well as antifungal activities than others. The rhodanine scaffold present in the synthesized compounds is expected to be a key part for imparting activity against microorganisms.

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